Proceedings of the Iowa Academy of Science

Volume 70 | Annual Issue Article 22

1963

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black granules, flowed actively in one direction only. There was no reversal during the entire 40 minutes.

Near the end of the period of observations, the active streaming slowed as if preparing to reverse its direction of flow, but there was only an irregular mixing which lasted for a few seconds, and then active streaming was resumed, in the same direction as before.

Because these plasmodia so rarely migrate through a clear space, I have been able to make confirmatory observations of the same length only once, but it both cases I observed the plasmodium continuously during a 40-minute period, and the presence of the black granules precluded mistaking the direction of flow. Both plasmodia were then kept under intermittent observation until they fruited.

Although *C. violacea* is a minute species like those which have been found to have protoplasmodia, its plasmodium differs from theirs in several respects. It spreads into a net, which protoplasmodia never do; it has an advancing fan-like portion, which they do not; and the streaming of the protoplasm is active and in veins, not irregular as in protoplasmodia. It is possible that the active streaming in this plasmodium also departs from the shuttle or reversible type considered characteristic of all myxomycetes.

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The Influence of Bicarbonate Ion Concentration on Cell Division and Cell Orientation of *Pediastrum*[1]

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Abstract: Disorientation of daughter colonies of *Pediastrum* was induced by reduction of the bicarbonate ion concentration in the culture medium. Threshhold values were established for disorientation by the use of two sources of bicarbonate ions. Below these threshhold values daughter colonies were disoriented; above them, oriented. When placed in new solutions having bicarbonate ion concentrations above the threshhold values, colonies which were disoriented by bicarbonate ion deficiency produced oriented daughter colo-

[1]Based on part of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the State University of Iowa.

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nies. When placed into new solutions having higher bicarbonate ion concentrations, colonies cultured in solutions with bicarbonate ion concentrations above threshhold values increased cell divisions in the mother cells and hence produced more cells in daughter colonies.

The algae used in this study came from a previous study (1). During that study I was unable to culture *Pediastrum* in Chu's (2) solution No. 11. While searching for suitable culture media, I discovered that the algae could be grown in a mixture of Chu's solution No. 11 with 10% river water. I shall term this mixture medium A. Colonies cultured in medium A with river water obtained in winter (Dec., Jan., Feb.) produced, contrary to expectations, only disoriented daughter colonies (Figures 1-5). Colonies cultured in medium A with river water obtained in the spring, summer, or autumn months were typically oriented (Figure 6). The unexpected disorientation of the *Pediastrum* colonies led to my present investigation.

The disoriented colonies were atypical in many respects. They were usually spheroidal and many-layered and unidentifiable as *Pediastrum*. Such disoriented colonies had been observed by Harper (3). He noted that they were produced by zoospores which were less motile than the zoospores forming oriented colonies. He attributed the disorientation to this lessened motility—"Weakened swarmspores with reduced activity are unable to achieve the typical compact plate form. . . . Weakened spores conform to the outline of the vesicle. . . and become oblong and irregular or many layered." Although his explanation seems plausible as far as it goes, Harper fails to account for this loss of vigor.

Methods and Materials

The author obtained a pure clonal culture of *Pediastrum boryanum* by isolating and removing the bacteria from a single colony.

A completely defined medium, designated as medium C, was produced by modifying Chu's (2) solution No. 11 (Table 1). Rodhe's (4) iron source was used. Stock solutions prepared with glass-distilled water were autoclaved for 20 minutes at 15 lb. pressure and stored in Pyrex flasks. The micrometabolite solution of Sager and Granik (5) was used.

The calcium bicarbonate solutions were prepared by placing 246 mg of anhydrous calcium carbonate in 600 ml of sterile glass-distilled water. Carbon dioxide was bubbled into the mixture until the solution was clear. If there were 100% conversion of the calcium carbonate by the carbon dioxide into calcium bicarbonate, 2 ml of the clear solution would contribute 1 ppm of bicarbonate ions in 998 ml of culture medium.

Table 1. Composition of nutrient medium C

Compound	mg per liter of compound in stock soln	ml of stock soln per liter of medium C
KNO_3	1000	40
$MgSO_4$	1000	25
K_2HPO_4	1000	5
$Cacl_2$	1000	7
Ferris Citrate + Citric Acid	100 + 100	10

Sodium bicarbonate solutions were prepared by dissolving 138 mg of sodium bicarbonate crystals in 100 ml of sterile glass-distilled water. If it is assumed that the sodium bicarbonate solution contributed only sodium and bicarbonate ions to the culture medium, one ml of this solution would contribute one ppm of bicarbonate ions in 999 ml of the culture medium.

The data of Tables 2 and 3 were based upon three assumptions: (1) there was 100% conversion of the calcium carbonate by the carbon dioxide into calcium bicarbonate, (2) the calcium bicarbonate solution contributed only calcium and bicarbonate ions to the culture medium, and (3) the sodium bicarbonate contributed only sodium and bicarbonate ions to the culture medium.

Temperature was automatically maintained at 21°±1°C. and was recorded continuously. Four 40-watt cool-white flourescent tubes in a white reflector 17 inches from the cultures supplied light at an intensity of approximately 350 ft. c. The cultures were lighted for 16 continuous hours daily, followed by 8 hours of darkness.

A pure inoculum established from the original pure culture was repeatedly subcultured in medium C, which had 15 ppm of bicarbonate ions from calcium bicarbonate. Thirteen day old cultures were used as inocula. They contained about 50,000 colonies per ml.

The experimental inocula cultures contained mainly colonies of uniform size which were not reproducing. The inocula cultures were combined, concentrated by centrifuging, and then washed and concentrated 3 times by adding 10 ml of sterile water, shaking, and centrifuging. Finally, distilled water was added to obtain inocula having approximately 30-45 thousand colonies per drop as determined with a hemocytometer.

For each experiment, 5 cultures were prepared by placing into each of 5 sterile test tubes (Pyrex, 15 x 180 mm) a 15 ml aliquot from a 100 ml solution containing sterile medium C and as much bicarbonate solution as was required for the particular experiment. The tubes were capped with cotton plugs and autoclaved for one minute at 15 lb. pressure.

After the test tubes had cooled to room temperature, hydrogen ion concentration was determined by use of a Beckman model G

pH meter. Tubes were inoculated with one drop of inoculum. The data of Tables 1 and 2 were obtained by counting all the algae in as many microscope fields as necessary (usually two) until approximately 100 colonies from each tube had been counted. The values obtained from the five tubes were averaged. The final pH reading was taken six days after the tubes were inoculated. In this period the algae had reproduced, and the young daughter colonies had reached approximately ½ of their maximum diameters.

Observations and Results

Table 2 shows the percent of disoriented daughter colonies in culture media containing various cencentrations of bicarbonate ions obtained from calcium bicarbonate. Note that the percent of disoriented colonies decreased with an increase of bicarbonate ion concentration. In fact, until a concentration of between 10-15 ppm of bicarbonate ions was reached, the percent of disoriented colonies definitely decreased with increasing concentrations. However, at bicarbonate ion concentrations higher than 10-15 ppm, the percent of disoriented colonies remained essentially constant. The pH values of these cultures increased from initial values of 6.8 - 7.3 to final values of 9.9 – 10.1. When daily pH data was taken, the greatest increase in pH occurred during the first four days after inoculation.

Table 2 also shows the percent of disoriented daughter colonies produced in culture media containing various concentrations of bicarbonate ions obtained from sodium bicarbonate.

Table 3 presents the variation in the number of cells in daughter colonies with various bicarbonate ion concentrations obtained from calcium bicarbonate. Ten ppm of bicarbonate ions was the least concentration in the culture media. Concentrations lower than 10 ppm were not used, because cultures at these concentrations always produced high percentages of disoriented daughter colonies whose cells could not be accurately counted. An increase in the amount of bicarbonate ions in the culture medium increased cell division and hence the number of cells per colony. Approximately 80% of the daughter colonies were 16-celled at all concentrations. However, with increasing bicarbonate ion concentration, the number of 8-celled daughter colonies decreased and the number of 32-celled daughter colonies increased.

Table 3 also presents the variation in the number of cells in daughter colonies with various bicarbonate ion concentrations obtained from $NaHCO_3$. With 5 ppm of bicarbonate ions, only a small percentage of all daughter colonies were disoriented. There was almost no disorientation at higher ion concentrations. With 5 and 10 ppm bicarbonate ions from this source, about

Table 2. The effect of the bicarbonate ion concentratoin on the disorientation of daughter colonies

Bicarbonate source	Concentration (ppm)	Percent Disorientation
$Ca(HCO_3)_2$	0	99.2
	5	91.4
	10	85.1
	15	16.4
	20	13.2
	30	16.5
	40	8.6
	50	7.4
	100	12.6
$NaHCO_3$	0	99.2
	5	16.4
	10	14.0
	15	4.4
	20	1.5

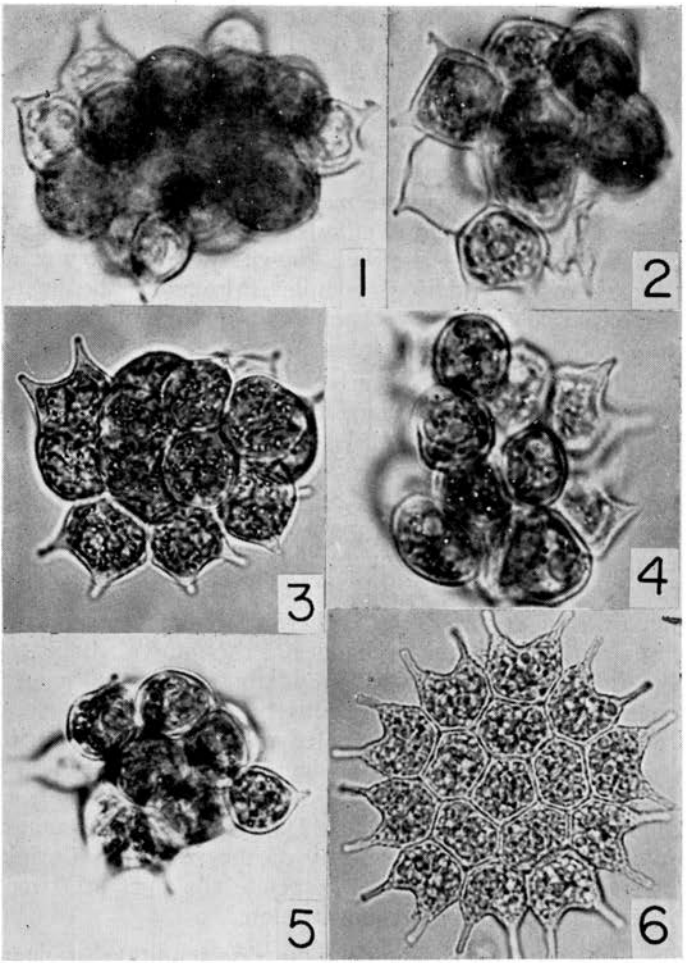
Table 3. The effect of the bicarbonate ion concentration on the number of of cells of daughter colonies

Bicarbonate source	Conc. (ppm)	%8 celled colonies	%16 celled colonies	%32 celled colonies	%64 celled colonies
$Ca(HCO_3)_2$	10	15.6	80.8	1.4	0
	12	9.9	88.2	1.9	0
	14	9.2	82.2	8.6	0
	16	8.7	80.6	10.6	0
	18	3.1	85.5	11.3	0
	20	1.2	70.4	28.4	0
$NaHCO_3$	5	0	57.5	42.4	0
	10	0	55.0	44.9	0
	15	0	13.7	84.0	2.5
	20	0	7.8	87.9	4.3

half of the daughter colonies produced were 16-celled and about half 32-celled. With 15 and 20 ppm most of the daughter colonies were 32-celled and a small percentage 64-celled.

A culture of disoriented colonies, obtained by growing the algae in medium C without bicarbonate, was used to inoculate tubes containing medium C with no bicarbonate and also tubes with medium C containing 20 ppm of bicarbonate ions from calcium bicarbonate. The daughter colonies produced from disoriented parent colonies in the tubes with 20 ppm of bicarbonate ions were identical to those produced from oriented colonies in 20 ppm of bicarbonate ions. That is, almost all of them were oriented. The daughter colonies produced in the tubes without bicarbonate from either oriented or disoriented parent colonies were almost entirely disoriented.

Bicarbonate ion concentrations below the critical amount did not appear to change the cell's ability to produce two horns. Many cells of disoriented colonies formed 2 horns (Figures 1-5). These cells are morphologically identical to those of oriented colonies.



Figures 1–5. Examples of disoriented colonies
Figure 6. A typical oriented colony

Reproducing colonies were carefully examined to determine the effect of bicarbonate concentration on zoospore activity. Zoospore activity was slight in cultures with no bicarbonate or in cultures whose bicarbonate concentration from calcium bicarbonate was below 10 ppm.

Discussion

Separate preliminary experiments with the culture medium in which the calcium ion (from $CaCl_2$) concentration and sodium ion (from NaCl) concentration were raised well beyond the maximum concentrations found in this study produced no noticeable effects on the algae. Other preliminary experiments in which *Pediastrum* was cultured in a fixed rnage of pH values

from 4 to 10 also produced no noticeable effects. This preliminary work seems to indicate that cell division and disorientation in *Pediastrum* do not depend upon the concentration of calcium or sodium or on the pH of the culture medium.

Although the computed values shown in the tables for bicarbonate ion concentrations may not represent the actual concentrations present in the culture media, it seems clear that cell division and disorientation in *Pediastrum* are affected by bicarbonate ion concentration. As is well known, the chemistry of aqueous bicarbonate solutions is complex. Moreover, the heat produced by autoclaving may change the bicarbonate ion concentration of the autoclaved solution.

The data suggest that bicarbonate ion concentrations below some critical amount produce disoriented daughter colonies. When cultured in media with less than these critical amounts of bicarbonate ions, *Pediastrum* produces daughter colonies whose cells are haphazardly grouped in a more or less spheriodal arrangement. The disoriented daughter colonies appeared identical to those produced in medium A containing river water obtained in the winter.

Bigeard (6) reported that spheroidal colonies were formed in response to aging and to changes of the mineral concentration of the environment. Harper (3) attributed atypically oriented colonies to reduced zoospore activity. My observations agree with Harper in that the disoriented colonies of my experiments were produced by zoospores of decreased motility, which were active for a shorter time than those producing typically oriented colonies. I further believe, with Harper, that disorientation is caused by the decreased motility of the zoospores during the swarming period. Moreover, I associate the decreased motility with low bicarbonate ion concentration.

Since this study suggests that the disorientation of daughter colonies is produced by a bicarbonate ion deficiency, it is necessary to adduce proof that the solutions of medium A containing winter river water had lower bicarbonate ion concentrations than those containing summer river water. According to Ruttner (7), water gets its bicarbonate supply by absorbing carbon dioxide as it percolates through soil rich in microorganisms and roots and then by dissolving calcium carbonate. Since in cold weather the life processes of roots and microorganisms are depressed, Ruttner's work suggests that river water collected during the cold seasons will be less rich in bicarbonate ion concentration than that collected during the warmer parts of the year. In the work which preceded this study the winter river water for medium A was obtained from the Iowa River at a time when the river, its tributary streams, and the surrounding land were frozen. Thus

we have evidence that the bicarbonate ion concentration in the river water used in this study was indeed lower in the winter than in the summer.

The results indicate that sodium bicarbonate is considerably more efficient than calcium bicarbonate in promoting cell division and cell orientation of *Pediastrum*. Further work has been planned to study this difference.

ACKNOWLEDGEMENT

I would like to express my appreciation to Prof. R. L. Hulbary for the help and advice relative to this work.

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Nematode Populations in Corn Plots Receiving Different Soil Amendments[1]

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Abstract: The magnitude of nematode populations in corn plots receiving manure, lime, or a fertilizer supplying N-P-K was compared with populations in corn plots not receiving manure, lime, or an N-P-K fertilizer. *Pratylenchus* spp. populations were highest in N-P-K fertilized plots and in manured plots. *Helicotylenchus microlobus* were highest in in plots in which no N-P-K had been applied. *Xiphinema americanum* populations were highest in limed plots. Seasonal population patterns for the three nematodes appeared to be characterized by two peaks, one in the early spring prior to the planting of corn, and the other in the late summer or fall related to the growth of corn. Only *Pratylenchus* spp. appeared to feed endoparasitically in corn roots.

Various parasitic nematodes have been associated with corn roots in Iowa. A primary step toward understanding the relationship of these nematodes to corn was to record the population changes that occurred throughout the year and to correlate these changes with associated factors.

It has been found that population patterns were usually related to the seasons (1, 2, 3). Climate and host-crop can also exert important influences on population patterns (1, 2, 4, 5, 6).

[1]Journal Paper No. J-4571 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1337.
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